Boelens and coworkers add to this flourish of XPF/Rad1/Mus81/Hef structural work by reporting the NMR solution structure of the heterodimeric complex between human XPF and ERCC1 C-terminal $(HhH)_2$ domains. The overall fold of the $(HhH)_2$ domains in human XPF/ERCC1 closely resembles that observed in other related HhH domain structures (Nishino et al., 2005b; Newman et al., 2005; Tsodikov et al., 2005), except for the observation that the second hairpin in the tandem HhH of XPF is replaced by a 3 residue β turn. The $(HhH)_2$ dimerization interface consists of largely hydrophobic residues and is anchored by two C-terminal Phe residues (XPF Phe 894, ERCC1 Phe 293) that bind into a hydrophobic pocket formed by the partner protein's HhH motif. The analysis of the dimerization interface corresponds to that reported in other work on human XPF/ERCC1 (Tsodikov et al., 2005; Choi et al., 2005).

What distinguishes the work of Tripsianes et al. (2005) from other structural investigations of human XPF/ERCC1 is the NMR chemical shift mapping of residues involved in stem-loop DNA binding, a DNA structural equivalent of the NER bubble. Perturbations of the chemical shifts of residues in the first and second hairpin motifs of ERCC1 were observed, but not to residues in the hairpin of XPF. Thus, the authors conclude that ERCC1 possesses the DNA binding activity and XPF the nuclease activity of the protein complex. Hence, combined with the recruitment of XPF, the DNA binding activity of ERCC1 serves to localize the XPF nuclease activity to the DNA lesion to allow for damage excision. Interestingly, this model differs from that proposed by Ellenberger and coworkers (Tsodikov et al., 2005), where it was observed that two molecules of ssDNA were required to saturate binding by one human XPF/ERCC1 $(HhH)_2$ heterodimer. These authors proposed that the $(HhH)_2$ domains of both XPF and ERCC1 bind to unpaired ssDNA strands of a bubble lesion. The difference in observed DNA binding functionalities for human XPF/ERCC1 $(HhH)_2$ heterodimer may be related to the different experimental conditions and DNA substrates used by the two groups.

Another critical finding of Boelens and coworkers is that the ERCC1 $(HhH)_2$ domain folds properly only in the presence of the XPF $(HhH)_2$ domain. This implies that XPF functions as a chaperone for ERCC1, and this result has a broader impact for NER research. Structural biology of NER proteins and complexes has been greatly hindered by the inability to produce intact recombinant materials at sufficient quantities. Stabilization of structural units via coexpression could well be an invaluable strategy for the study of a variety of NER complexes.

Although the power of the domain approach is clear, several challenges lie ahead before the ultimate goal of developing a mechanistic understanding of NER at the atomic level can be achieved. One critical step involves placing domains into a more complete structural context, which includes not only the tertiary structure of the intact proteins but also an appreciation of how these proteins interact with each other. There is also a critical need to integrate results from static snapshots with data that inform the dynamic transitions between structural states. Now that the first steps have been made, the stage is set for an exciting future that will lead to a fundamental understanding of the structural mechanisms of the multiprotein assemblies in NER.

Christopher G. Bunick[1] and Walter J. Chazin[1]
[1] Biochemistry Department
and Center for Structural Biology
Vanderbilt University
Nashville, Tennessee 37232

Selected Reading

Choi, Y.J., Ryu, K.S., Ko, Y.M., Chae, Y.K., Pelton, J.G., Wemmer, D.E., and Choi, B.S. (2005). J. Biol. Chem. *280*, 28644–28652.

Friedberg, E.C. (2001). Nat. Rev. Cancer *1*, 22–33.

Lehmann, A.R. (2003). Biochimie *85*, 1101–1111.

Newman, M., Murray-Rust, J., Lally, J., Rudolf, J., Fadden, A., Knowles, P.P., White, M.F., and McDonald, N.Q. (2005). EMBO J. *24*, 895–905.

Nishino, T., Komori, K., Ishino, Y., and Morikawa, K. (2003). Structure *11*, 445–457.

Nishino, T., Komori, K., Tsuchiya, D., Ishino, Y., and Morikawa, K. (2005a). Structure *13*, 143–153.

Nishino, T., Komori, K., Ishino, Y., and Morikawa, K. (2005b). Structure *13*, 1183–1192.

Riedl, T., Hanaoka, F., and Egly, J.M. (2003). EMBO J. *22*, 5293–5303.

Tripsianes, K., Folkers, G., Ab, E., Das, D., Odijk, H., Jaspers, N., Hoeijmakers, J., Kaptein, R., and Boelens, R. (2005). Structure *13*, this issue, 1849–1858.

Tsodikov, O.V., Enzlin, J.H., Scharer, O.D., and Ellenberger, T. (2005). Proc. Natl. Acad. Sci. USA *102*, 11236–11241.

Structure, Vol. 13, December, 2005, ©2005 Elsevier Ltd All rights reserved. DOI 10.1016/j.str.2005.11.002

In Silico Access to the Nuclear Pore Complex

Translocation of biomolecules through the nuclear pore complex is governed by interactions that occur between phenylalanine-glycine-rich nucleoporins and transport receptors. Using molecular dynamics simulations, Isgro and Schulten (2005) replicate and predict these interactions with startling spatial clarity and temporal detail.

The concerted interplay of biomolecules drives elementary biological processes with a spatial and temporal complexity that continues to elude the most advanced experimental techniques in biology. However, this may

not be a concern for much longer, since these "gaps" are increasingly being filled with the aid of theoretical and computational studies. By combining the rapidly increasing trove of new biological structures with the rigorous mathematical tools of physics, theoretical biophysicists are now able to gain deep insight into the mechanisms of biological function by implementing advanced computing strategies in the form of molecular dynamics (MD) simulations (Humphrey et al., 1996; Kale et al., 1999). Indeed, the pioneering work of Schulten and coworkers typifies the merits that MD simulations can bring to visualizing complex biological processes in action (Tajkhorshid et al., 2002).

In this issue of *Structure*, Isgro and Schulten (2005) turn their attention to the nuclear pore complex (NPC), the supramolecular machinery representing the sole gateway for cargo exchange between the nucleus and the cytoplasm (Fahrenkrog and Aebi, 2003). NPCs are large assemblies (i.e., ~120 MDa in the case of vertebrate and ~60 MDa in the case of yeast NPCs) that consist of nuclear pore proteins, termed nucleoporins or Nups, of which ~30% harbor phenylalanine-glycine (FG)-repeat sequence domains that are natively unfolded (Denning et al., 2003). FG-nucleoporins are strongly implicated in mediating nucleocytoplasmic transport of receptor bound cargoes (or receptor-cargo complexes) by means of transient binding interactions with transport receptors such as, for example, the import receptor importin-β (Bayliss et al., 2000, 2002).

As a definitive first in the field, Isgro and Schulten shed light on this process by replicating three of the four known binding sites of FG-repeat segments to importin-β through MD simulations. And just to add the much-required "kick" to in silico experiments, the predictive power of their MD work is convincingly displayed by discovering a fourth binding site that has only recently been verified experimentally (Liu and Stewart, 2005)—that is, independently and only after completion of the present MD investigation.

Isgro and Schulten go on to provide compelling evidence that five additional binding spots may exist between FG-repeat segments and importin-β. Besides revealing an ample number of distinct hydrophobic patches on the surface of importin-β that represent the primary binding sites for FG-repeat motifs, their discovery suggests that the extensive ability of importin-β to interact with FG-Nups may offset the entropic cost of being confined to the NPC. Conversely, a lack of binding interactions may imply that it is not energetically favorable for "passive" molecules, i.e., non-receptor-bound cargoes, to be targeted to the NPC. Most significantly, the authors also report that their simulations reveal interactions occurring between FG-repeat segments which are not involved in binding interactions with importin-β within the time scale of the simulation, i.e., ~30 ns. Although in this case the simulated FG-repeat segments are relatively short, i.e., made up of either 4 or 12 residues, it is nonetheless an important observation that implies that FG-repeats can exhibit cooperative behavior over time (Ribbeck and Gorlich, 2002).

Taken together, these findings highlight several aspects of cargo translocation through NPCs that remain speculative and unresolved. One of the controversies surrounding the field involves the "paradoxical" mechanism behind the selective gate/permeability barrier in NPCs (Rout et al., 2000; Ben-Efraim and Gerace, 2001; Ribbeck and Gorlich, 2002). While FG-Nups promote receptor-mediated transport, it is alleged that their collective behavior also functions as a substantial barrier to non-receptor-bound molecules; i.e., non-receptor-bound cargoes end up being repelled from the vicinity of the NPC due to the presence of the high-density FG "barrier." While small molecules such as water and ions can passively diffuse through the nuclear pores, the NPC poses a restrictive barrier to the passage of larger molecular cargoes when not in complex with a transport receptor. The same barrier does not seem to perturb receptor bound cargoes which are efficiently "funneled" through the nuclear pore. Moreover, since receptor-mediated transport rates seem to be extremely rapid even for large cargoes, it becomes apparent that the premise of the NPC sorting mechanism is not solely based on size exclusion.

From this discussion, perhaps future MD simulations can be applied to resolving the following questions:

1) How does a receptor bound cargo interact with FG-repeats so as to promote translocation without hindering it?
2) Are there varying degrees of binding affinities between FG-repeat segments and different transport receptors?
3) How significant are inter-FG interactions after taking into account the entropic behavior of natively unfolded FG domains? What are the relevant time scales?
4) What constitutes the underlying gate-barrier mechanism, and how is non-receptor-bound cargo prevented from traversing the NPC?
5) How do changes in environmental conditions within the vicinity of a NPC affect these interactions?

This work illustrates the need to apply additional meaningful and sensitive experimental approaches to investigating nucleocytoplasmic transport. In this context, the potential of MD simulations lies in their ability to unveil relevant molecular effects not yet accessible to experiment. Bearing in mind that nucleocytoplasmic transport is governed by dynamic molecular interactions (i.e., biomolecules moving and interacting over tens of nanometers in nanoseconds to several milliseconds), it challenges experimentalists to assay and understand NPC function at the nanoscale, i.e., at the mesoscopic level. Most important in this endeavor, the key components guiding nucleocytoplasmic transport need to be understood on such *relevant* length and time scales to be able to test the computational results. For example, ascertaining the mechanics of individual and surface-tethered clusters of FG-nucleoporins at the molecular level can provide a more rational understanding of how they behave in the local interaction zone (i.e., where near-field physics typically comes into play) of an individual NPC. Perhaps only by more rigorously correlating computational, physical and biochemical findings with each other will a more rational and comprehensive picture

of NPC function and nucleocytoplasmic transport start to unfold.

Roderick Y.H. Lim[1] **and Ueli Aebi**[1]
[1]Maurice E. Müller Institute for Structural Biology
Biozentrum
University of Basel
Switzerland

Selected Reading

Bayliss, R., Littlewood, T., and Stewart, M. (2000). Cell *102*, 99–108.

Bayliss, R., Littlewood, T., Strawn, L.A., Wente, S.R., and Stewart, M. (2002). J. Biol. Chem. *277*, 50597–50606.

Ben-Efraim, I., and Gerace, L. (2001). J. Cell Biol. *152*, 411–417.

Denning, D.P., Patel, S.S., Uversky, V., Fink, A.L., and Rexach, M. (2003). Proc. Natl. Acad. Sci. USA *100*, 2450–2455.

Fahrenkrog, B., and Aebi, U. (2003). Nat. Rev. Mol. Cell Biol. *4*, 757–766.

Humphrey, W., Dalke, A., and Schulten, K. (1996). J. Mol. Graph. *14*, 33.

Isgro, T.A., and Schulten, K. (2005). Structure *13*, this issue, 1869–1879.

Kale, L., Skeel, R., Bhandarkar, M., Brunner, R., Gursoy, A., Krawetz, N., Phillips, J., Shinozaki, A., Varadarajan, K., and Schulten, K. (1999). J. Comput. Phys. *151*, 283–312.

Liu, S.M., and Stewart, M. (2005). J. Mol. Biol. *349*, 515–525.

Ribbeck, K., and Gorlich, D. (2002). EMBO J. *21*, 2664–2671.

Rout, M.P., Aitchison, J.D., Suprapto, A., Hjertaas, K., Zhao, Y.M., and Chait, B.T. (2000). J. Cell Biol. *148*, 635–651.

Tajkhorshid, E., Nollert, P., Jensen, M.O., Miercke, L.J.W., O'Connell, J., Stroud, R.M., and Schulten, K. (2002). Science *296*, 525–530.